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Beerling, D.J., Osborne, C.P. and Chaloner, W.G. (2001) Evolution of leaf-form in land plants linked to atmospheric CO2 decline in the Late Palaeozoic era. *Nature*, 410 (6826). pp. 287-394. ISSN 0028-0836

<https://doi.org/10.1038/35066546>

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Evolution of leaf-form in land plants linked to atmospheric CO₂ decline in the Late Palaeozoic era

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The widespread appearance of megaphyll leaves, with their branched veins and planate form, did not occur until the close of the Devonian period at about 360 Myr ago. This happened about 40 Myr after simple leafless vascular plants first colonized the land in the Late Silurian/Early Devonian^{1,2}, but the reason for the slow emergence of this common feature of present-day plants is presently unresolved. Here we show, in a series of quantitative analyses using fossil leaf characters and biophysical principles, that the delay was causally linked with a 90% drop in atmospheric p_{CO_2} during the Late Palaeozoic era^{3,4}. In contrast to simulations for a typical Early Devonian land plant, possessing few stomata⁵ on leafless stems, those for a planate leaf with the same stomatal characteristics indicate that it would have suffered lethal overheating, because of greater interception of solar energy and low transpiration. When planate leaves first appeared in the Late Devonian and subsequently diversified in the Carboniferous period, they possessed substantially higher stomatal densities⁶. This observation is consistent with the effects of the p_{CO_2} on stomatal development⁷ and suggests that the evolution of planate leaves could only have occurred after an increase in stomatal density, allowing higher transpiration rates that were sufficient to maintain cool and viable leaf temperatures.

The origin of megaphyll leaves was an important event in land plant evolution with a major impact on a wide variety of terrestrial biogeochemical and ecological processes. Indeed, planate leaves are the basis of primary productivity for virtually all terrestrial life, especially tetrapods and insects. The fossil record unequivocally demonstrates that early vascular land plants in the Late Silurian/Early Devonian were either leafless, possessing short, cylindrical, aerial stems or bore only minute enation leaves (microphylls)². Over the next 40 Myr a progressive evolutionary sequence followed. The early axial-type plants showed a gradual shift, developing much-

branched determinate lateral systems ('proto-leaves') and then fully 'webbed' (that is, laminate) planate leaves with dichotomous venation by the Late Devonian¹. The long delay in this relatively simple evolutionary development is surprising given the ubiquity of the laminate leaf form in modern plant groups, its importance in photosynthetic carbon acquisition, and that the same interval of time witnessed the structurally far more complex evolution of vascular plant life cycles from homospority to the seed habit^{2,8}. This critical, but neglected, feature of land plant evolutionary biology has remained enigmatic since it was reported descriptively as the 'telome theory' over 70 years ago⁹. Megaphyll planate leaves became abundant in Late Devonian/Early Carboniferous fossilized terrestrial floras¹⁰, but first appeared in the Early Devonian plant *Eophyllophyton bellum*¹¹, indicating that the production of laminate leaves was possible, although not widespread, at this time.

Here we address this evolutionary problem by simulating organ gas exchange rates and energy budgets (see Methods) to quantitatively assess the probable costs and benefits of evolving a planate leaf. We first consider a simple erect axis and planate leaf operating in the Early Devonian atmosphere (Fig. 1), both with the low stomatal density characterizing plant fossils of this age (Table 1). Planate leaves at low latitudes intercept over 200% more solar energy than axes and, coupled with high stomatal resistance curtailing transpiration, this raises leaf temperatures well into the range for lethal damage observed in extant tropical taxa (50–55 °C)¹². Even at higher latitudes, where the solar angle is lower, temperatures reach 40 °C, a value close to the limit for photosynthetic CO₂ uptake¹². In contrast, an axis maintains low transpiration rates but avoids overheating by minimizing the surface area presented to intercept solar energy during the middle of the day. Given that axis rates of photosynthesis exceed those of surviving planate leaves, no selective advantage in terms of carbon gain is conferred by evolving this leaf form. Even if planate leaves had evolved with a high stomatal density, the resulting transpiration rates (9–13 mmol H₂O m⁻² s⁻¹) would only have been sufficient to cool leaf temperatures to 38 °C at low latitudes. These rates require xylem conductances of over ten times those measured¹³ for *Psilotum nudum*, a plant possessing a primitive stele with close anatomical similarities to early Rhyniophytes. Such transpiration rates are clearly therefore incompatible with the functioning of early land plants. However, the higher rates for an erect photosynthetic axis fall well within its hydraulic constraints.

The earliest preserved fossil megaphyll leaf cuticles date to the Carboniferous and have higher stomatal densities than early land plant axes by one to two orders of magnitude (Table 1). Geochemical models show a major draw-down in p_{CO_2} between the Early

Table 1 Leaf and environmental characteristics used in biophysical modelling

Leaf characteristics					
Type of leaf	Stomatal density* (mm ⁻²)	Stomatal pore length/width (μm)	Diameter of axis/leaf width (mm)	Diffusion path length (μm)†	$V_{\text{max}}/J_{\text{max}}$ (μmol m ⁻² s ⁻¹)‡
Low-density axis	10	40/20	4	500	11/48
Low-density planar leaf	10	40/20	60	100	11/48
High-density planar leaf	1,000	20/10	60	100	78/157
Midday palaeoenvironmental conditions§					
Environment	Atmospheric CO ₂ (Pa)¶	Atmospheric O ₂ (kPa)¶¶	Air temperature (°C)#		
			20° S	60° S	
Early Devonian	300	21	35	30	
Late Devonian/Carboniferous	30	30	35	30	

* The low values are representative of Late Silurian/Early Devonian plant fossils, and the high values are representative of Carboniferous plant fossils^{5,6,27}.

† Taken as 0.125 of axis diameter and 0.5 of leaf thickness, assuming a hypostomatous leaf.

‡ From ref. 22, where V_{max} and J_{max} are the maximum rates of carboxylation activity and photosynthetic electron transport respectively²³.

§ All simulations were made for conditions representative of still clear summer days with solar energy interception values calculated as in Methods, a relative humidity of 60% and a wind speed of 0.5 m s⁻¹, a value for conditions close to the ground surface, where small Early Devonian axes would have evolved planate leaves.

¶ From ref. 3.

¶¶ From ref. 14.

From land-surface summer temperature computed by a general circulation model for a pole-centred supercontinent in the Carboniferous³⁰. We have conservatively assumed no warming of summer temperatures in the high-CO₂ Early Devonian atmosphere, compared to the low-CO₂ Carboniferous atmosphere, and have not accounted for a diurnal temperature increase over mean monthly global climate model temperatures.

Devonian and Late Devonian/Early Carboniferous and a rise in the atmospheric p_{O_2} (refs 3, 14), both associated with increased organic carbon burial by the spread of Devonian forests, the formation of Carboniferous swamp lands, and plant-enhanced chemical weathering of rocks. Simulations for Late Devonian/Carboniferous leaves (Table 1) indicate transpiration rates sufficient to cool leaf temperatures well below the lethal range, even in the warm low latitudes (Fig. 1). The reduced stomatal resistance of these planate leaves benefited photosynthetic productivity under the unusually low atmospheric CO_2/O_2 ratio by increasing CO_2 diffusion into leaf mesophyll and thereby minimizing photorespiratory CO_2 evolution through the carbon oxidation pathway¹⁵.

We tested our biophysical simulations of ancient land plants by first computing their water-use efficiencies (carbon gain per unit of water lost) using modelled gas exchange characteristics. These were next compared with independent estimates obtained using the stable carbon isotope composition ($\delta^{13}\text{C}$) of fossilized Palaeozoic terrestrial organic matter⁴ and a well-validated model¹⁶ relating plant water-use efficiency to $\delta^{13}\text{C}$ (see Methods). Calculated in this way, both approaches consistently show similar values and a pattern of declining plant water-use efficiency between the Late Silurian period (410–415 Myr ago) and Carboniferous (300–292 Myr ago) (Fig. 2). Agreement with fossil evidence therefore provides qualitative and quantitative support for our modelled fluxes of CO_2 and H_2O between photosynthetic organs and the surrounding atmosphere.

Development of photosynthetic structures with high stomatal

densities, and correspondingly higher transpirational cooling capacities, was probably an essential requirement for the evolution of laminate leaves. Observations on a range of plant species show that atmospheric p_{CO_2} regulates leaf stomatal development, with the potential for genetic adaptation on a timescale of millions of years¹⁷. Therefore, the drawdown in atmospheric p_{CO_2} in the Late Palaeozoic era and the concurrent observed increase in stomatal density, is a likely ancient example of this effect of CO_2 on plant development. In this context, however, the increased stomatal density had important consequences for their subsequent morphological evolution. Moreover, a 40-Myr delay between the axial form of Late Silurian/Early Devonian land plants and the development of megaphyll planate leaves is consistent with the timescale required to remove CO_2 from the atmospheric reservoir by silicate rock weathering and organic carbon burial³.

The likelihood of high-temperature injury and collapse of photosynthetic productivity in Early Devonian plants will have been critically dependent upon the ultimate lobe width of planate photosynthetic structures, because this strongly influences boundary layer resistance and convective heat dissipation, that is, cooling capacity¹⁸. We calculate that widths of 50–100 mm at high latitudes and less than 20 mm in equatorial regions are maximal before temperatures increase and photosynthesis drops abruptly (Fig. 3). The earliest 'proto-leaves' should therefore have been rather narrow, especially for plants distributed in warm tropical regions, and this model prediction is supported by two pieces of evidence from the fossil record. Observations made on Early Devonian (Emsian) plant fossils at a palaeolatitude of $\sim 15^\circ\text{S}$ showed that some of the earliest megaphyllous, but non-laminate, leaves were finely divided (7–10 mm in width)¹⁹. The earliest yet reported laminate megaphyllous leaves with branched venation were very small and finely divided (up to 2–5 mm broad)¹¹, dated to the Early Devonian (Pragian);

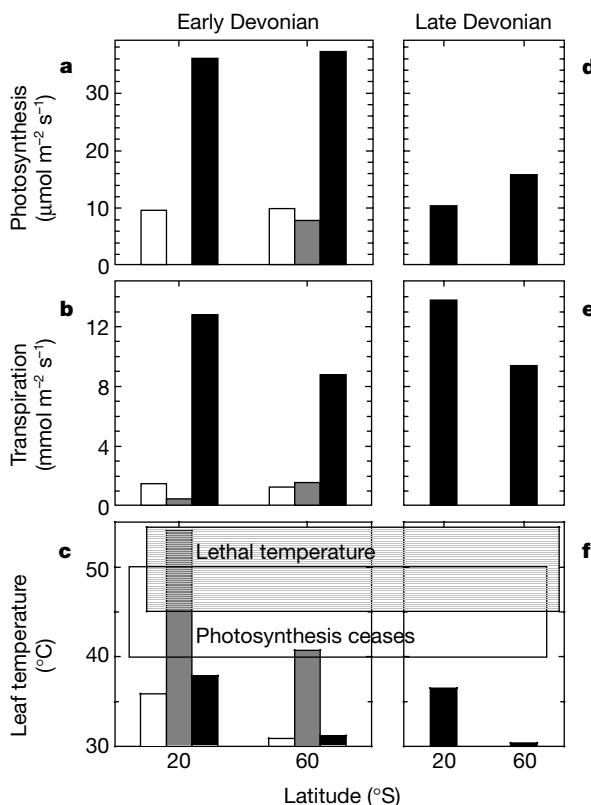


Figure 1 Simulated biophysical properties of leaves and axes in the Late Palaeozoic environment. Midday photosynthetic rates (**a**), transpiration rates (**b**) and temperatures (**c**) for an axis with a low (white) stomatal density and planate leaves with high (black) or low (grey) stomatal density operating in a high- p_{CO_2} Early Devonian atmosphere (Table 1) at 60°S and 20°S . The absence of the low stomatal density planate leaf in **a** is because its temperature is lethal (see **c**). **d–f**, Adjacent boxes show the same ecophysiological traits for a planate leaf with a high stomatal density in the lower p_{CO_2} atmosphere of the Late Devonian. Horizontal boxes in **c** and **f** indicate the temperature range at which CO_2 uptake by photosynthesis ceases and the lethal range for extant warm temperate and tropical species¹².

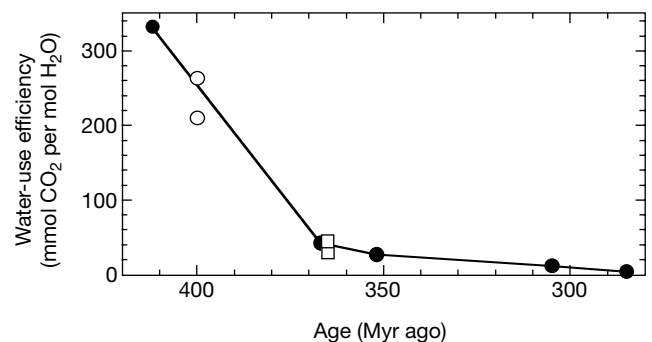


Figure 2 Stimulated and isotopically derived changes in leaf and axis water-use efficiency during the Late Palaeozoic era. Black circles, estimate from soil organic $\delta^{13}\text{C}$; white circles, simulated Early Devonian axis; white squares, simulated Early Carboniferous planate leaf.

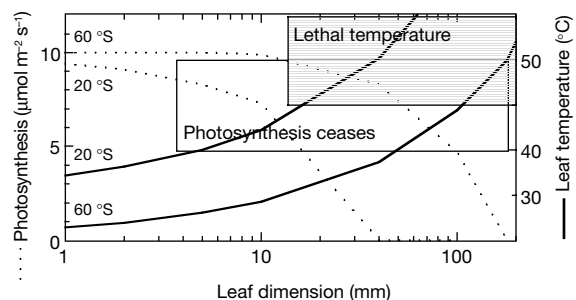


Figure 3 Simulated biophysical properties of Early Devonian planate leaves (Table 1) in relation to width. Dotted line, photosynthesis; solid line, leaf temperature, shown for two palaeolatitudes.

they belonged to *E. bellum* growing at a palaeolatitude of $\sim 25^\circ$ N. It follows that the energetic constraints precluding the evolution of wider laminate leaves would have been more relaxed in the cooler climates of the mid- to high latitudes, pointing to these regions as the likely geographical sources for this leaf form.

As the atmospheric p_{CO_2} fell during the Late Palaeozoic, we expect the laminate leaf to have become more widespread in the plant kingdom and progressively larger or less dissected. By the close of the Devonian, plants from several groups possessed planate leaves with an extensive dichotomizing venation. Some of these had megaphyll leaves up to 30–40 mm across, with a morphology similar to *Ginkgo*, and are assigned to several ill-defined genera including *Ginkgophyllum*, *Platyphyllum*, and *Psymphyllum*. The genus *Enigmophyton* Hoeg., probably of Middle Devonian age, had *Ginkgo*-like leaves up to 160 mm long and 120 mm broad. These examples constitute the earliest known occurrences of sizable laminate leaves in the fossil record¹ and clearly indicate that after 40 Myr, biotic and abiotic changes allowed a greater interception of solar energy without causing lethal temperature damage to the leaf, or significant high-temperature limitation to photosynthesis. An important consequence for terrestrial plants able to support planate leaves with high transpiration rates (Fig. 2) would have been the selective pressure driving improved water and nutrient transport, crucial for plants as their heights rapidly increased from the Middle Devonian onwards¹⁰. However, by the Middle to Late Devonian, fossilized plant assemblages reveal that vascular systems were probably more efficient²⁰, and water uptake from the soil improved by the evolution of deep rooting systems²¹. □

Methods

Simulations were made using a fully coupled photosynthesis/stomatal resistance/energy balance model, accounting for the feedbacks between changes in the environment and their influence on gas exchange²². Fossil stomatal geometry was used to calculate minimum stomatal resistance. We then allowed for the effects of CO_2 , leaf-to-air vapour pressure deficit, irradiance and temperature on stomatal pore width, and used this modified stomatal resistance to calculate the leaf energy budget. Photosynthetic rate was predicted with a steady state biochemical model of leaf CO_2 assimilation²³, with a feedback modifying the intercellular CO_2 concentration of the leaf which, in turn, influenced stomatal opening and thus changes in leaf energy balance.

V_{max} and J_{max} (Table 1) were derived by calculating the maximum rate of photosynthesis and corresponding values of intercellular CO_2 level from stomatal resistance using the fossil stomatal characteristics^{24,25}. In this way, the entire process of modelling leaf gas exchange was based on the fossil record and was independent of physiological initialization using data from plants growing in the present climate and CO_2 . These estimates are necessarily approximate, but have no effect on our conclusions regarding thermal damage to leaves in the Early Devonian. We note that the gene coding sequence, protein structure and kinetic properties of the key photosynthetic enzyme Rubisco are highly conserved among disparate taxonomic groups of C_3 plants²⁶, indicating little or no change in its properties throughout plant evolutionary history.

Within the coupled model, generalized responses of stomatal pore widths to the vapour pressure deficit, CO_2 and irradiance were used and, although there is some uncertainty attached to extrapolating these back to extinct plants, they are nevertheless representative of a wide range of modern taxa. All plants avoid desiccation under dry air conditions and in bright sunlight by closure of stomatal pores, as included in our model. In addition, anatomical studies of the guard cells of Devonian axes have shown that their form is remarkably close to that in modern plants²⁷, which suggests that they probably operated in a similar manner. Influences of the environment derived in the model from today's experiments simply modify the minimum stomatal resistance, calculated from the geometry and density of fossil stomata, rather than setting stomatal resistance itself.

Interception of solar energy by photosynthetic organs was computed from geometric considerations, with the zenith angle of planate leaves taken from reconstructions of permineralized Devonian plants ($\sim 40^\circ$ from the horizontal)¹ and integrated for all azimuth angles²⁸. Our model accounts for the greater distance travelled by CO_2 molecules from the atmosphere to the sites of carboxylation in an axis compared to a planate leaf (Table 1) by calculating total axis/leaf resistance to CO_2 diffusion (r_i , in units of s m^{-1}) using:

$$r_i = r_s + r_i + r_w + r_a$$

where r_s is the stomatal resistance to CO_2 diffusion of the leaf/axis, r_i and r_w are the diffusion resistances through the intercellular spaces, and across the cell wall to the site of photosynthesis (including plasmalemma, cytosol, mesophyll and chloroplast membrane) respectively. r_i is dependent upon the length of the diffusion pathway from the atmosphere to the site of photosynthesis (see Table 1). Because of the obvious difficulty of calculating r_w from fossil materials, we assumed a constant value for both axes and laminate leaves from measurements on modern plants of 240 s m^{-1} (ref. 18). The final term, r_a , is the boundary layer resistance which depends on wind speed and leaf width¹⁸ (see Table 1 for

leaf dimensions and environmental conditions). The calculated value of r_i consists of a combination of data obtained from measurements on fossil plant materials and assumed characteristics from extant plant leaves. However, r_i is dominated by r_a and r_s , and these are the two components best constrained by measurements on fossils.

The water-use efficiency of Palaeozoic plants was calculated with the isotopic composition of atmospheric CO_2 used during photosynthesis ($\delta^{13}\text{C}_a$), and measurements of the isotopic composition of terrestrial organic matter ($\delta^{13}\text{C}_b$)⁴, to calculate discrimination against ^{13}C (Δ) from $(\delta^{13}\text{C}_a - \delta^{13}\text{C}_b)/(1 + \delta^{13}\text{C}_b/1,000)$ (ref. 16), after correcting $\delta^{13}\text{C}_b$ to account for the difference between litter and leaf tissue values ($\sim 3\text{‰}$)²⁹. Plant water-use efficiency was calculated as $p_{\text{CO}_2} \times [1 - (-\Delta + a)/(-b + a)]/1.6$ where a is fractionation associated with diffusion (4.4‰), b is fractionation associated with Rubisco (27‰) and 1.6 is the ratio of gaseous diffusivities of CO_2 and water vapour in the air¹⁶. Modelled water-use efficiencies were calculated from the ratio of intercellular to atmospheric p_{CO_2} (c_i/c_a ratio) derived from the gas exchange data (Fig. 1) and used to calculate Δ as $a + (b - a) \times c_i/c_a$. This value was then used to calculate the water-use efficiency as for the carbon isotope data.

Received 29 August 2000; accepted 15 January 2001.

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Acknowledgements

We thank D. Edwards, J. A. Raven, F. I. Woodward and G. R. Upchurch for helpful comments and discussion on the manuscript. D.J.B. gratefully acknowledges funding through a Royal Society University Research Fellowship and the Natural Environment Research Council, UK.

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